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Interference by Hydroxyethyl Starch Used for Vascular Filling in Latex Agglutination Test for Cryptococcal Antigen

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The glucuronoxylomannan component of the *Cryptococcus neoformans* capsular polysaccharide confers serotype specificity, and its detection in cerebrospinal fluid or serum by the latex agglutination test is used for diagnosis. Low-molecular-weight hydroxyethyl starches can be used as an alternative to albumin for vascular filling. This study reports the occurrence of a false-positive result with the Pastorex *Cryptococcus* test (Sanofi Diagnostics Pasteur, Marnes la Coquette, France) for a patient receiving hydroxyethyl starch characterized by a substitution ratio of 0.6 (Elohes, Biosedra, Sèvres, France).

Cryptococcus neoformans is a yeastlike encapsulated fungus responsible for life-threatening infections in immunocompromised patients (13). The main component of the *C. neoformans* capsular polysaccharide is a glucuronoxylomannan (GXM). Five serotypes have been described (A, B, C, D, and A-D) on the basis of the specificity of polyclonal antibodies to whole cells of *C. neoformans* (10). Antigenic structures intrinsic to the GXM allow the distinction between serotypes (15).

An important tool in the diagnosis of cryptococcosis is the latex particle agglutination test (LPAT), which uses latex particles coated with an anti-GXM antibody to detect capsular polysaccharide antigen in the serum or cerebral fluid. The LPAT is a rapid and easy procedure for the diagnosis of cryptococcosis; however, several investigators have noted that false-positive reactions can occur. The presence of a rheumatoid factor was the most common cause of this phenomenon (3, 9). Agar syneresis fluid gave false-positive reactions (6). Interferences due to noncryptococcal infections, such as *Capnocytophaga canimorsus* (16) or *Trichosporon beigelii* (12), and nonspecific reactivity in human immunodeficiency virus-infected patients (17) have also been demonstrated. Several procedures have been proposed to reduce false-positive reactions with the LPAT: (i) pretreatment of specimens with heat (7); (ii) incorporation of latex particles coated with normal rabbit globulin (3); and (iii) use of pronase (8), dithiothreitol (9), or 2-mercaptoethanol (17).

In anesthesia and intensive care procedures, low-molecular-weight hydroxyethyl starches (HES) are an alternative to albumin (2). Two of these modified polysaccharidic polymers are used in the Anesthesia-Reanimation Unit of the Besançon University Hospital, HES-0.6 and HES-0.45 (Elohes and Lomol, respectively; Biosedra, Sèvres, France). These HES are characterized by substitution molar ratios of 0.6 and 0.45 (i.e., the ratio of hydroxyethyl ether molecules to glucose molecules) and average molecular masses of 200,000 and 250,000 Da, respectively (1).

Immunosuppressive treatment instituted after transplants can be a predisposing factor for cryptococcosis. Therefore, the LPAT for *C. neoformans* antigen is performed in our laboratory in the routine follow-up of hepatic transplantation. This article reports a case of a false-positive reaction in the Pastorex *Cryptococcus* test (Sanofi Diagnostics Pasteur, Marnes la Coquette, France) due to interference by HES-0.6 in a recipient of a liver transplant.

Cryptococcal antigen test. The Pastorex *Cryptococcus* test uses latex particles coated with an anti-GXM monoclonal antibody (5). The LPAT was performed according to the manufacturer's recommendations. All specimens were treated with pronase for 30 min at 56°C. Samples that gave a positive agglutination reaction were serially diluted 10-fold to determine the endpoint of agglutination.

Case report. The patient, a 63-year-old male with cirrhotic hepatitis B, underwent a hepatic transplantation on 20 July 1994. After the operation, he was admitted to the Reanimation Unit of the Besançon University Hospital. Vascular filling was performed with fresh-frozen plasma, and posttransplantation treatment included the parenteral administration of nutritients (glucose, amino acids, lipids, vitamins, and trace elements), dopamine, insulin, immunosuppressors (cyclosporin, methylprednisolone, and azathioprine), and antibiotics (piperacillin and ciprofloxacin). The patient also received gamma globulin therapy against hepatitis B virus. On 21 July, the same treatment was administered except that HES-0.6 (500-ml perfusion) and 4% albumin were used for vascular filling. The LPAT was performed as a routine test, and a positive reaction was obtained with serum and urine sample titers of 10^6 and 10^1, respectively. However, there were no clinical symptoms to indicate a cryptococcal infection. Nevertheless, the patient was given fluconazole therapy (400 mg/day, intravenously). The next day, a new serum sample gave a positive LPAT with a lower titer (10^1), and an assay performed on the urine sample was negative. A final LPAT performed 10 days later on a serum sample was negative. Fluconazole therapy was stopped. During this period, all direct examinations of the cerebrospinal fluid by india ink techniques and all urine, cerebrospinal fluid, and blood cultures were negative.

LPATs on low-molecular-weight HES. The Pastorex *Cryptococcus* test was applied to HES-0.6 and HES-0.45 solutions. Tenfold serial dilutions of HES-0.6 were prepared in buffer solutions. The assay for *Cryptococcus* antigen was positive with titers from 10^1 to 10^6. The pure solution produced a negative result, which could be explained by the occurrence of a zone

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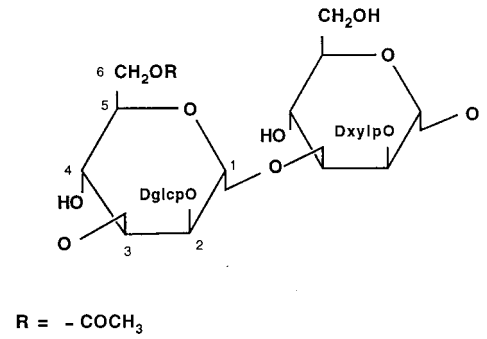

FIG. 1. Structure of GXM from *C. neoformans* serotype A.

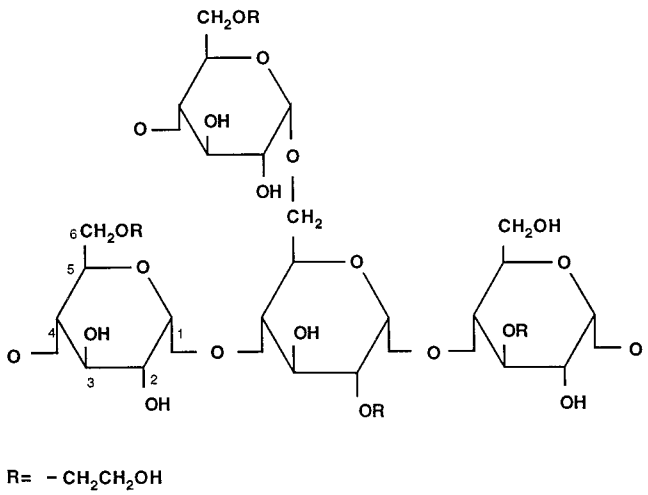

FIG. 2. Structure of HES.

phenomenon. The HES-0.45 solution was tested in the same way. Regardless of the dilution, all the results were negative.

Pure and diluted solutions of HES-0.6 gave negative results when tested against the Crypto LA test (Wampole Laboratories, Cranbury, N.J.), which is a polyclonal antibody-based latex agglutination kit.

The Pastorex *Cryptococcus* test uses an anti-GXM monoclonal antibody (E1) to sensitize the latex beads. E1 was produced in mice by immunization with unmodified capsular polysaccharide purified from *C. neoformans* serotype A (5). The GXM of serotype A isolates consists of a linear α (1-3)-linked mannan backbone singly substituted with nonreducing D-xylosyl and D-glucuronosyl acid groups; *O*-acetyl groups are present at C-6 of the mannosyl residues (Fig. 1) (4). Subtle differences involving the relative proportion of xylose and glucuronic residues, the degree of mannan substitution, and the percentage of *O*-acetyl attachments seem to be responsible for the distinctions among the five serotypes identified by reactions to immune sera. The precise target of E1 activity has not been totally elucidated (5), but the glucuronyl and *O*-acetyl groups are considered the major immunodeterminants of serotype A GXM (14).

The risk of viral disease transmission has restricted the use of fresh-frozen plasma in vascular filling. This policy has led to an increased use of albumin. Low-molecular-weight HES are newly commercially available plasma substitutes that can be used as an alternative to albumin (2). These natural macromolecules, which are extracted from corn, consist of two polymers, amylose (5%) and amylopectin (95%), and are modified by hydroxyethylation of amylopectin. The substitution affects elimination kinetics by protecting the molecule from hydrolysis by ubiquitous α-amylase. With the appropriate substitutions of hydroxyethyl radicals on C-2, C-3, or C-6 of the glucose units (Fig. 2), hydrolysis is reduced and the duration of efficacy is prolonged, thus providing better characteristics for clinical use. Degradation by α-amylase is followed by renal excretion of the metabolic products. HES have a variety of molecular weights, and elimination half times vary from 7 h to 5 days, depending on the sizes of the molecules. With HES-0.6, about 97% of the initial concentration is eliminated within 9 days (11).

The LPAT performed on the HES-0.6 solution was positive, whereas it was negative with the HES-0.45 solution. This observation indicates that the molar substitution ratio influences the molecular structural characteristic necessary for antibody binding. The appropriate number and localization of hydroxyethyl residues on the starch molecule may provide a structural requirement for the integrity of the epitopes recognized by the E1 antibody. A positive reaction of the Pastorex *Cryptococcus* test was obtained with a sample from a patient who received HES-0.6 for vascular filling after a liver transplant. No clinical symptoms indicated cryptococcosis, and all direct examinations and cultures of cerebrospinal fluid, urine, and blood were negative. We conclude that the presence of HES-0.6 in the blood caused a false-positive reaction with the Pastorex *Cryptococcus* test. This interference was not reduced by pretreatment of specimens with heat and pronase. It would not have been eliminated by the use of 2-mercaptoethanol or dithiothreitol because the interfering substance is not a protein but a polysaccharide. An LPAT performed on serum 10 days after the infusion was negative. This delay corresponded to the timetable for the elimination of HES from the patient. Such interference was not found with the Crypto LA test. Epitopes recognized by polyclonal antibodies might be different from those bound by E1. Further studies to test HES against other latex agglutination kits widely used in several countries are in progress.

Despite procedures used to reduce false-positive results, interference factors still present a problem when clinical specimens are tested for the presence of cryptococcal antigen. Cases of interference by HES must be reported because of the increasing use of such polysaccharidic molecules for vascular filling. In the present case, the titer was so high that discussion with clinicians prompted an investigation of the possibility of a false-positive LPAT result. However, a lower titer would have not been recognized as interference. This observation confirms that LPAT results should be interpreted and used only within the context of clinical information.

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